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Protein Disorder in Dynein Regulation by Dynactin and NudE
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Cytoplasmic dynein is a multi-subunit protein complex responsible for retrograde transport of diverse cellular cargoes along microtubules. Dynein is comprised of heavy chains responsible for motor activity, and intermediate chain (IC) and light chains for cargo attachment and regulation. Dynein light chain LC8 is conserved across species and its binding promotes dimerization and stabilization of IC but its effect on dynein regulation remains unclear. Dynein activity is regulated by other proteins such as dynactin, which is essential for most dynein activities; and NudE, which functions in targeting dynein to the kinetochores. LC8, dynactin subunit p150Glued and NudE coiled-coil domain all bind the disordered N-terminal domain of IC. Using NMR and ITC on Saccharomyces cerevisiae and Drosophila melanogaster constructs, we show that the N-terminal helix of IC is a single α-helix (SAH) instead of a dimeric coiled-coil as predicted. NMR titrations map the exact residues of IC involved in binding to both p150Glued and NudE and the accompanying changes in structure and dynamics, while ITC experiments identify the domains of IC necessary for full binding affinity. In yeast, p150 and NudE both bind the IC SAH domain, and both interactions are enhanced when LC8 is present. In Drosophila, p150 and NudE both bind to the SAH domain but p150 also binds to a second site on IC, a nascent helix separated from the SAH domain by a 4-residue linker. A dimeric IC formed by cross-linking IC chains has increased affinity to p150 but similar affinity to NudE, indicating that bivalency causes differential effects on binding regulatory proteins, and illustrating intriguing species variation in dynactin binding to IC. These studies illustrate the importance of multiple techniques to elucidate interplay of order and disorder in providing both structural and functional versatility for complicated systems.

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A Fuzzy DNA Binding Region in MBD2 Recruits the Histone Deacetylase Core Complex of NuRD and Modifies Kinetics of DNA Binding
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The MBD2 protein recruits and assembles the Nucleosome Remodeling and Deacetylase (NuRD) complex and thereby uniquely combines binding specificity for methylated DNA with histone deacetylation and chromatin remodeling. This complex has been implicated in methylation dependent silencing of genes during development and aberrant silencing of tumor suppressor genes during carcinogenesis. We have focused on structural and biophysical analyses of MBD2 with the long-term goal of developing methods to disrupt formation of the MBD2-NuRD complex. Along these lines, we have previously characterized and determined the structures of the coiled-coil and methylcytosine binding (MBD) domains of MBD2. More recently we have characterized an intrinsically disordered region (IDR) of ~120 amino acids linking the MBD and coiled-coil. NMR chemical shift analyses, CD, and AUC show that this region, MBD2(IDR), does not adopt a regular structure in isolation or in the context of full-length protein. Yet the MBD2(IDR) stably binds three proteins that form the histone deacetylase core of NuRD. We show that the first two-thirds of the MBD2IDR are necessary and sufficient to bind the histone deacetylase core while mutating two consecutive within this region is sufficient to abrogate binding to the core complex and disrupt the function of MBD2 in cells. At the same time, adding the MBD2(IDR) to the MBD2(MBD) in vitro modifies DNA binding primarily by reducing the observed off rate and increasing affinity by ~100 fold. We find that the MBD2(IDR) does not adopt a regular fold in the presence of DNA thereby functioning as a fuzzy DNA binding region. Together our studies show that the IDR of MBD2 plays a dual role both augmenting DNA binding affinity and recruiting a large portion of the NuRD complex.

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C/EBPβ: Case Study for the Importance of Intrinsic Disorder for Protein Function
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The basic region:leucine zipper (bZIP) DNA-binding protein, C/EBPbeta is a key regulator of numerous cellular processes, but can also contribute to tumorigenesis and to viral diseases. It binds to specific DNA sites as homo- or heterodimers and interacts with other transcription factors to control transcription of a number of eukaryotic genes. Importantly, C/EBPbeta induces chromatin opening at several cell-type specific enhancers.
C/EBPbeta is an intrinsically repressed protein that is activated in response to growth factors. This report discusses possible mechanisms modulating the biological activities of C/EBPbeta based on results from sequence analysis, molecular modeling, X-ray crystallography and mutagenesis studies. Analysis of primary structure indicated that C/EBPbeta is natively unstructured protein, which consists of regions with potential to fold upon binding to molecular partners and regions that retain irregular conformations independently of their environment. Conformational flexibility allows for the initial auto-inhibition via intramolecular interactions, and subsequently facilitates formation of transient intermolecular interactions that regulate C/EBPbeta's dimerization, nuclear translocation, DNA- binding and trans-activation activities in response to cellular signals.

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Structural and Dynamic Analysis on Disordered H4 Histone Tail by Modified AWSEM-MD
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DNA compaction in eukaryotic cells is mediated by positively charged octamers comprised of histone proteins. The latter consists of well folded core segments, that come together to form a central cylinder, and flexible tails, protruding out from cylinder's rim. Despite being disordered, histone tails play an important role in bridging interactions between neighboring nucleosomes, regulating folding structure and dynamics of chromatin fibers. Histone tails, in turn, are mainly regulated via post-translational modifications, such as methylation and acetylation at various positions. Because of their flexibility and disordered nature, it has been difficult to investigate histone tails both computationally and experimentally. In particular, it is desirable to develop coarse-grained, yet accurate models of histone tails, such that subsequent nucleosomal and polynucleosmal simulations could be carried out within feasible times. To achieve this goal, we added new interactions to the associative memory, water mediated, structure and energy model (AWSEM-MD), which is typically used for folding of globular proteins or binding studies. We found that modified AWSEM-MD reproduces well the complex conformational ensemble of the H4 histone tail, obtained from atomistic simulations with explicit solvent. In particular, the cumulative and site-specific effects of various acetylation combinations are consistent with the all-atom results. Our proposed extension of AWSEM-MD may allow simulating intrinsically disordered proteins with high accuracy and computational efficiency.

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The Acetylation Landscape of the H4 Histone Tail
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The DNA of higher organisms wraps around histone proteins to form the basic unit of chromatin, the nucleosome. Each histone has N- and C-terminal tails that protrude outward from the nucleosomal surface, beyond the surrounding DNA. Histone tails, which are intrinsically disordered, play an important regulatory role for genetic processes, and, because of their high flexibility, they are difficult to characterize experimentally. Furthermore, histone tails exhibit a diverse array of post-translational modifications that alter their structure and dynamics, as well as their interactions with DNA and other proteins. We investigate the effects of increasing the degree of acetylation on histone tail H4 by performing extensive explicit solvent all-atom molecular dynamics simulation. We explore the conformational preferences of wild type, mono-, di-, tri-, and tetra-acetylated H4 histone tails. Our results demonstrate that the effects of acetylation on the H4 histone tail are both cumulative and site-specific.

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Comparing Solution Structures of Amylin and CGRP by Nanosecond Laser-Pump Spectroscopy and Atomistic Simulations
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Amylin and calcitonin gene-related peptide (CGRP) are intrinsically disordered proteins, members of the calcitonin (Ct) peptide family. They are found with